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Identification of Piroplasma of Dogs, of the Type Piroplasma canis.  
Transmission of the French Strain by the South African Tick, Haemaphysalis leachi. Doubtful Value of the Proofs of Crossed Immunity  
With the Piroplasmoses.

by E. Brumpt.

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Ann Parasitol 16:2:97-116, 1938.

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The transmission of canine piroplasmosis by the acarids, natural vectors, and vicarious experimental vectors, which I began to study in 1910, is a subject which required numerous breedings of new and infected ticks, which are both difficult to obtain and difficult to maintain (1). That is why I could only make known my first endeavors in this area in 1919 and 1922. From this time, thanks to helpful assistance from the Rockefeller Foundation, then from the Conseil Supérieur de la Recherche Scientifique, which assigned to me a technical assistant, it has been possible for me to organize, with better method, a certain number of findings concerning the transmission of piroplasmosis in different domestic animals: dogs, sheep, oxen.

Canine piroplasmosis, besides its practical importance, since it causes the death of a great number of animals exported into regions where it is endemic, presents, from the scientific viewpoint, a considerable interest, especially in approaching the various problems involved in the identification of species, active acquired immunity, the conditions determining the virulence of the germs, and finally the action of the vector hosts on the differences in infection power and virulence of the parasites.

The base upon which to establish the species morphologically identified in Protozoology are often quite uncertain. The study of Piroplasmae of the type P. canis points up the difficulty of identifying germs grouped under this term which have certain different antigenic properties. These last make it difficult to interpret the proofs of artificial crossed immunity by inoculations of blood or natural tick bites, because the observed facts can not be explained simply by a variation of virulence in the strains.

Certain authors, it is true, would seem to hold that there are several strains within the type P. canis, perhaps even several species which have arisen due to the action of the different host vectors to which they have become adapted over the centuries. I can not bring myself to accept this opinion, because, as I mentioned in 1934 and 1937, certain spirochetes, for example the S. duttoni, have retained the same biological properties, although transmitted in nature by two very different ornithodoroses (O. moubata and O. erraticus) (2), and also because, to give an opposite example, the virus of Rocky Mountain military fever transmitted by the same species of tick (Dermacentor andersoni), present, according to region, a quite variable virulence. Other similar examples could be cited, particularly with the pathogenic trypanosomes and their host vectors (Brumpt, 1925).

The studies which I have undertaken on the different piroplasmoses of domestic animals being far from complete, and in danger of being interrupted for several reasons, I thought it well to publish here and now the fragmentary results that are available so these findings could be more rapidly placed at the scrutiny of other authors.

I. Transmission of the French Strain by the South African Tick,  
Haemaphysalis leachi.

Canine piroplasmosis from P. canis can be transmitted naturally beyond doubt by three species of ticks, and experimentally by a fourth species.

In South Africa, Lounsbury (1901-1904) established experimentally the role of H. leachi; Christophers (1907), Brumpt (1919), then James, cited by Wenyon, and this last author (1926), have shown that in the Indies and in Tunisia, Rhipicephalus sanguineus the normal vector of the canine infection. In 1919, the transmission of piroplasmosis around Paris by Dermacentor reticulatus was demonstrated by my experiments; finally the vicarious role of Dermacentor andersoni was discovered in studies which I made with my student F. Larrouse.

Identification of strains. Artificial crossed immunity. -- The different strains transmitted naturally by the three species of ticks mentioned above, have been studied, as to the immunity conferred by one strain against another, by inoculation of blood by several authors.

Laveran and Nattan-Larrier (1913), using Professor Nuttal's South African virus, conserved at Cambridge since 1904 by passage in dogs, and similarly a French virus from around Paris isolated later, showed that out of seven dogs which had acquired the immunity against the French virus, six died as witnesses. Inversely, two dogs immunized against the South African virus, and inoculated with the French virus contracted light infections and were cured.

Other authors' experiments consequently established that the two strains seem distinct. In effect, if the African virus simply belongs to a more active strain than the French virus, the experiments with crossed immunity would be otherwise: the dogs having immunity for the French virus would have been infected with the South African strain but would have presented attenuated infections, contrary to what was the case; on the other hand the dog having acquired immunity against the stronger South African virus would have had a complete immunity against the French virus, which was not the case. Also Laveran and Nattan-Larrier were led to conclude that the African canine piroplasmosis (Cambridge strain, 1904) constitutes, if not a species, at least a variety quite distinct from the French canine piroplasmosis around Paris.

Likewise in 1913, Ciuca showed that a dog cured of piroplasmosis originating in Tonkin (3) but which was no longer in a state of premunition, for his blood was no longer infectious, succumbed to an inoculation of the S. A. virus (Cambridge strain, 1904) given 28 months after the first infection.

In 1919, in the "communication" where I noted the conditions under which the Tunisian canine piroplasmosis is transmitted by the tick Rh. sanguineus, I published the case of a young dog (B.47, II), inoculated with the blood of another dog (B.46, II), infected with the Tunisian strain after being bitten by the Rh. sanguineus, on 6 June 1911, and which, inoculated with the virus from Tonkin, on 21 October, when he was in perfect health and apparently in a state of premunition (4), died eight days later with numerous parasites. This case is particularly interesting for it seems to show that, for the two viruses transmitted by the same species of tick, it is possible to have a deadly super-infection, by inoculation of blood, in certain cases at least.

The following observation shows that an animal, cured for several days of a natural infection of P. canis of Moroccan origin, could die following inoculation of blood containing Piroplasmae of the South African strain.

Dog 287/XXII. -- Age, several months. Feb. 2, 1937, app. 50 Rh. sanguineus adults (No. 181/XXI) fixed themselves on him. The eleventh day, Piroplasmae appeared in the blood and reached a maximum of 20 parasitized erythrocytes at the extremity of the smears on the 13th day, the date the temperature rose to 40°. Parasites were present up to the 29th day. The 31st day, the animal, clinically cured for 15 days, was inoculated with 1 cc. of blood from a subject (556/XXII) infected with the South African strain. The dog died on the 8th day, after having presented on the 7th day, around 60 parasitized erythrocytes per field and strong hemoglobinuria.

The first infection with the Moroccan piroplasmosis, thus had not given any premunition.

- II. Is a partial immunity acquired by animals being successively in a state of premunition against the three strains: French, Moroccan, and South African, as transmitted by their normal vectors?

History. -- Before continuing with my experiments on this subject, let me point out that the first facts, dealing with the study of a part of this problem, were published by Reichenow (1935) who, in causing a dog (while presenting a chronic infection of P. canis, from the Far East, transmitted by Rh. sanguineus) to be bitten by Dermacentor reticulatus adults carrying a French piroplasmosis, caused a new infection stronger than the first. It is true that the same author notes in his conclusions, that he has succeeded in transmitting three times to the same dog (5) the same strain of canine piroplasmosis by Rh. sanguineus at intervals of a few weeks. Let us note that this dog had been treated with acaprine (= sothelone), after the first and second infection and that he was perhaps no longer in a state of premunition. I must add, nonetheless that, according to my experiments, following a single dose of acaprine, the animals are clinically cured, but remain in a state of premunition. Thus it was that dog 186/XXIV, treated the 10th day after the start of the experiment, presented a parasitic and feverous (40°) relapse on the 18th day, and that the blood of a dog (228/XXIV), cured by sothelone turned out to be infectious thirty days later for dog 497/XXIV.

Personal Observations. -- Dog 66/XXIV. -- Age app. 4 months. Feb. 5, 1938,

this animal is placed in a sack with app. 40 adult Rh. sanguineus, taken from a sixth generation of ticks always raised on a stubborn host, the hedgehog, but having retained, nonetheless, their infectious power. The acarids fixed themselves on various parts of the body. Jan. 14, three first females of Rh.'s drop off and the next day the Piroplasmae appear in the blood. Jan. 17, since the animal had about 10 parasites per field, he received an injection of sothelone which cured him. Jan. 25, this dog whose temperature is normal is replaced in a sack with 20 females of D. reticulatus (exp. 185/XXIV). The ticks fix themselves rapidly and from 1-b. 2-9, 9 half-gorged females (6) fall off and are collected from the panel placed under the cage. This dog, instead of presenting as he did the first time a fever of 40-40.4°, has a temperature of only 39.4°. Feb. 3, 9 days after the start of this second experiment, Piroplasmae appear in the blood and are found until the 24th day, with a maximum of 2 or 3 infected erythrocytes per field on the 10th day.

This animal not having been clinically sick during the course of this second experiment and no longer presenting parasites by direct examination of the blood, is submitted to a third trial and on Feb. 23, about 50 Haemaphysalis leachi, infected by the South African strain (exp. 371/XXIV) are placed on him. His temperature, taken each day, rises from the 5th day to 40.4°, which is caused not by Piroplasmae which appear on the 8th day only, but to an infection by a new sanguicolous spirochete, transmitted by the tick H. leachi, the identification of which I will soon publish with my technical associate M. Marchad.

From the 8th to the 13th day, the Piroplasmae, many of which are phagocyted by the large mononucleuses of the peripheric blood increase and reach the point of infecting 15 erythrocytes per field on the 13th day, when the temperature rose to 40.0° C. 24 gorged female Haemaphysalis are collected from the 8th to the 16th day. The 17th day temperature is normal, but the 18th day, it drops to 35° C., and this same day, Sunday March 13th, the dog died between 1430 hours and 1500 hours. He was placed immediately in the refrigerator and the autopsy made the next day revealed hemoglobinuria and an extremely intense infection of the organs. In the kidney, in particular, the infected erythrocytes reached the level of 90 out of 100.

All during the infection determined by the Haemaphysalis, the parasites are always very numerous in the blood without causing the reappearance of the spirochetes observed in large number the 5th day.

This experiment shows that the two anterior infections with the Moroccan and French strains have not given any premonition against the last infection, during which the Piroplasmae were particularly abundant. I will point out, nonetheless, that this third infection, which ended in death has lasted 18 days after the placing of the infectious ticks rather than 12 to 15 days, as is most frequently the case.

Dog 186/XXIV. --- New subject app. 12 months old, control for the preceding dog for the infection by the D. reticulatus females. Jan 25, 1938, this animal is placed in a sack with 20 female ticks; he presented parasites the first time on the 13th day and, on the 14th day, there are observed seven parasitized erythrocytes in 100 microscopic fields. The later examination were negative. Temperature rarely exceeded 39° C.; the infection was milder than that of 66/XXIV, although this last had already had an attack of Tunisian piroplasmosis. 4 half-gorged ticks were collected the 17th and 19th days.

Feb. 25, 1938, this dog was again placed in a sack with about 50 Haemaphysalis leachi, transmitting the South African piroplasmosis (exp. 414/XXIV). Although the dog did not have any fever, the South African Piroplasmae appeared from the 7th day, but always less numerous; their maximum: 1-3 per field, only attained on the 10th day, when the temperature rose to 39.4 C. The animal received then a dose of zothelone and the Piroplasmae disappeared the second day following, but the 18th day a feverous relapse occurred, the temperature rose to 40°, and the parasites appeared quite numerous in the erythrocytes.

25 gorged female ticks were collected between the 7th and 14th days.

In a few days, this dog will be bitten by the Rh. sanguineus infected with the Moroccan strain of canine piroplasmosis, to complete the experiments with surinfection published in this work.

Dog 437/XXIV. — App. 1 year old. Control for dogs 66/XXIV and 186/XXIV. March 3, 1938, 50 adult H. leachi of infectious breeding 63/XXIV are placed on this animal and he presented Piroplasmae on the 9th day, with a temperature of 40.2°. The South African piroplasmosis followed its normal course and the animal died from this infection on the 16th day.

### III. Transmission by ticks of new breedings (the habitual vector of other strains) of strains of canine piroplasmosis adapted to a particular vector.

The first experiment which seems to have been attempted on this point is the following, which I published in 1919(b):

1) The South African virus and Rh. sanguineus. — About 60 adult Rh. sanguineus, obtained from new nymphes gorged on a dog infected with a strain of South African piroplasmosis (Cambridge strain, 1904), preserved through passages through several years, are placed on a dog (274/III) and did not give him any infection. The reason for this is perhaps that the virus, passed on artificially from dog to dog for 10 years, had lost the property of evolving among ticks.

2) French virus from the vicinity of Paris and Rh. sanguineus. In 1931, Nieschulz and Wawo-Roentoe did several experiments using a French virus sent to them by Kikuth (7) and a new breeding of Rh. sanguineus, originating from Java.

After having nourished some new adult ticks on dogs infected with piroplasmosis, they placed several thousand larvae on a splenectomized dog (156), then more than 100 Rhipicephalus adult sons on dog 157, also splenectomized, without result.

A second series of experiments, in which 300-400 nymphes coming from 32 females nourished on a very infected dog, were placed on dog 195, equally gave only negative results.

Finally, the adults coming from 120 new nymphes nourished on a parasitized dog, placed on dog 207, did not cause any infection (8).

The Holland authors attribute their successive failures to the fact that the canine piroplasmosis studied by them was conserved from dog to dog for too long a time.

In 1932, Regendanz and Reichenow confirmed the observations of Nieschultz and Wawo-Roentoe and, in addition obtained negative results in using the same breeding of Rh. sanguineus and a French strain which happened to be transmitted to a dog when bitten by a D. reticulatus. But these new rhipicephales were able to be infected with Reichenow's strain of canine piroplasmosis from the Far East, which establishes their aptitude to transmit normally certain canine Piroplasmae.

In conclusion of his various experiments, Reichenow (1935) conjectures that the Piroplasmae transmitted by the tick Rh. sanguineus, should be separated from the classical P. canis and be considered as a species different from the one he calls Babesia major.

3) The French virus from around Paris and Haemaphysalis leachi. -- I have succeeded in transmitting the French piroplasmosis with some adult H. leachi, infected in the nymph stage on a dog having a natural infection determined by the D. reticulatus. These are the details.

a) Genealogy of the non-infectious breeding of H. leachi. -- The larvae of new ticks (lots 361, 362 and 363, XXII) sent from Pretoria by Du Tout, Director of the Institut d'Onderstepoort, were placed March 5, 1937, on dog 575/XXII, age around 3 months, and he was not infected. The nymphs coming from these larvae, placed the 20th of April following, on two new dogs 1269/XXII and 885/XXII, did not infect them. It is in gaining numerous larvae, enclosed in eggs laid by gorged females on these last two dogs, that I have gained a very important new breeding which I have used for various experiments.

b) Infection of the new ticks with various Piroplasmae. -- Some larvae (719/XXII), placed on a hedgehog, allowed us to obtain some new nymphs which were divided into two lots. The first was placed on a dog (1167/XXIII) having a strong infection of P. gibsoni of Chinese origin: after they became adults, they were placed on a dog (188/XXIV), on Jan. 25, 1938, and had not caused any infection 6 days later (9). On the other hand, this dog, in not presenting any infection of P. canis, can thus be considered as the control for the following experiments:

The second lot of nymphs (24/XXIV), was placed, Dec 28, 1937, on a young dog (1122/XXIII), bitten previously on Dec. 20 by some D. reticulatus adults (934/XXII), which had transmitted to him the French piroplasmosis from the vicinity of Paris (10) (Strain 274/XVII).

c) Infection of new dogs. -- The adult ticks coming from the nymphs of this second lot were placed half and half on the two dogs 287 and 288/XXIV.

Dog 287/XXIV. -- Animal around 3 or 4 months old. App. 200 H. leachi, half of lot 24/XXIV, attached themselves on him between Jan. 8 and Jan 9, 1938. The ticks gorged themselves normally, the dog died on the 5th day, after the normal incubation period for piroplasmosis. All of the Haemaphysalis collected after the death of this animal as well as those, partially gorged, which were removed

from the body of dog 288/XXIV, were placed in a sack with a new dog 311/XXIV, on whom they fixed themselves rapidly.

Dog 288/XXIV. -- Animal about 4 months old. Feb. 8, the other half of lot 24/XXIV of H. leachi fixed themselves on him. Feb. 15, the first gorged female dropped off and to prevent the dog's death from exhaustion all the embedded ticks are removed and transplanted in part onto dog 311/XXIV. Feb. 17, the examination of the blood is positive and the temperature reaches 39°.

In order not to let the dog have too strong an infection of piroplasmiasis, he received an injection of sothelone. Feb. 18, the animal, already quite weak when the treatment was given was found dead. The autopsy revealed a strong renal infection (11).

Dog 311/XXIV. -- Animal app. one year old. Feb. 14, 1938, all the H. leachi dropped or removed from dogs 287 and 288/XXIV were placed with this animal in a sack and for the most part they fixed themselves on the dog. Starting Feb. 16, the gorged females began to drop off. The examination of the blood is negative from the 6th up to the 16th day. Immediately thereafter, from March 2, the receptivity to French piroplasmiasis, which the dog would be able to contract by the transplantation of H. leachi, is tested by the application on its body of 50 infectious D. reticulati (443/XXIV). Mar. 9, on the 7th day, the Piroplasmae appeared and the temperature reached 41°C., and the number of infected erythrocytes was 2 to 3 per field; the 9th day, the number was up to 2 to 5 infected erythrocytes per field; the dog was not treated with sothelone and the illness followed its course. This last control experiment shows that the transplantation of ticks had not given any infection to this animal (12).

Once cured, this dog will finally be infected successively by the Rhipicephalus and the Haemaphysalis.

d) Attempted infection by daughter-ticks, uncertain vectors, bred from females nourished on a dog taken by French piroplasmiasis.

Dog 368/XXIV. -- Mongrel about one year old. On Feb. 22, 1938, this animal was placed in a sack with daughter-ticks of H. leachi, bred from ticks nourished on dog 458/XXIII, infected by the bite of D. reticulatus 763/XXII, and died on July 14, 1937. The larvae and nymphes bred of these females had been nourished on ground-hogs.

The blood of this dog examined up to Mar. 10, remained negative. On this last day some infectious Dermacentor's (763/XXII) were placed on him (exp. 504/XXIV), and seven days later the first Piroplasmae appeared in the periphéric blood. This positive result established the receptivity of the dog to the strain of French piroplasmiasis, although the ticks used on Feb. 22, had not transmitted it to him.

e) Transmission from stage to stage. -- The experiments to transmit the French canine piroplasmiasis by adult H. leachi, infected during the nymphal stage are especially interesting to report since Lounsbury has had negative results with nymphes of the same species, infected hereditarily by the South African canine strain.

On the other hand, I have noted, on my side, in the publication (1919a) in which I established the roll of the tick, D. reticulatus in the natural transmission of canine piroplasmosis around Paris, only negative results. I will briefly outline the unfruitful attempts to transmit from stage to stage which I undertook:

a) The following experiments show that the new larvae of D. reticulatus nourished on virulent blood (Paris strain, 109/V), were unable to transmit the sickness, neither during the nymphal nor the adult stage.

Experiment 122/V. -- Hexapode larvae, bred from 18 uninfected females (63/V) were placed Mar. 25, 1919, on a greatly parasited dog (110/V). The 200 nymphs placed on a young dog (exp. 142/V), on April 11, did not infect him. The 15 adults of these nymphs still surviving on Oct. 8, were placed on a dog (exp. 463/V); the dog was not infected. Inoculated 10 days later, with blood from dog 462/V (French strain 109/V), he took sick on the fifth day and died on the seventh.

b) The following experiment shows that the new nymphs of D. reticulatus nourished on virulent blood, did not transmit the infection when they reached the adult stage.

Experiment 145/V. -- New nymphs, coming from different breedings were placed, March 24, on virulently infected dog (110/V) April 2, the 5 adults (3 males and 2 females), coming from this breeding fixed themselves on a young dog (141/V) and did not infect him. Due to the small number of adults available, this experiment is hardly demonstrative.

It follows from these findings that the canine piroplasmosis finds a favorable environment for its evolution only in the adult tick.

In 1935, Shortt, in an important work which he published on the evolution of P. canis, pointed out according to his experiments that the transmission from stage to stage does exist. Larvae or nymphs infected on dogs can transmit the piroplasmosis to the next stage. However, given these facts which I have established (1937, 1938) concerning the conservation of the virus for 6 generations in Empicaphali raised on obstinate hosts and the lack of details furnished by Shortt and even more so by Christophers, on the genealogy of ticks considered new, which these authors used, a different interpretation from theirs is here proposed.

Toward completing the facts exposed in this article, I believe it would be good to give below the experiments published up to this time:

1) on the infectious stages of Ixodidae, natural vectors of canine piroplasmosis.

2) on the vicarious experimental hosts of different strains of P. canis.

#### IV. INFECTIOUS STAGES OF TICKS BRED FROM FEMALES NOURISHED ON DOGS PRESENTING PIROPLASMAE IN THEIR BLOOD.

Relating to the infectious stage of different Ixodidae, natural vectors of canine piroplasmosis, the result of Lounsbury's experiments (1901-1904) is that the larvae and nymphs coming from infected female H. leachi are unable to transmit piroplasmosis to the dog, which only could be done by the daughter-ticks.

In the Indies, Christophers (1907) working with the tick Rh. sanguineus, has shown that if the larvae coming from infected females do not transmit the disease, it is sometimes a result of the bite of nymphs. Shortt (1900) confirmed these facts.

My experiments with these same species of Ixodidae, a certain number of which had been infected with a Tunisian strain of piroplasmosis were sent from Tunis by L. Blaisot, in 1910, have allowed me to establish the exclusive roll of the adult daughter-ticks. As I wrote in 1919:

"About 50 female adults taken from a dog (475/I), whose blood was virulent, gave thousands of larvae. These larvae, placed on three dogs (480/I, 481/I, 483/I), did not give them the disease. The nymphs bred from the preceding larvae were placed on five dogs (482/I, 483/I, 492/I, and 494/I), on which were collected respectively about 2,200, 600, 600, 125, and 125 nymphs gorged with blood; none of the dogs was infected. The adults, coming from the preceding nymphs, approximately six weeks hatched, and placed on dog 482/I, in December, refused to attach themselves, which is normal for this hibernating species. In May these same adults, placed on dogs B46/II and B48/II, gave to the first a mortal infection (360 females collected), to the second a mild infection.

"The hexapod larvae, born of this new generation of adult daughters having infected the two dogs B46/II and B48/II, were raised on the two dogs B78/II and B79/II which did not show any infection. The numerous ticks to which these larvae gave birth, placed on the two dogs B84 and B85 did not infect them any further, and we are forced this time again to the adult ticks to be able to give the disease to dog 180/II.

"Concerning the infectious stage of the tick D. reticulatus, the following experiments show that the larvae coming from infected females do not transmit the piroplasmosis, a fact already demonstrated by the observations of Nocard and Motas (1902).

"Experiment 191/IV. -- May 19, 1919, app. 80,000 larvae, collected during the middle of November 1918, bred from 40 females infected with the French virus (109/V of March 18, 1919) (Exp. 111/V), placed on the young dog (141/V), did not infect him, although more than 400 gorged larvae had been collected.

"Because none were available, no dog was bitten by the nymphs (13) coming from the infected females.

"The adult daughter-ticks, coming from females nourished on a parasited dog, transmitted the disease. (exp. 462/V).

"From the observations, it is concluded that the Piroplasmae can only take their yet unknown infectious metacyclic form, in the adult daughter-tick. The larvae and nymphs must harbor evolutionary elements of a morphological and physiological nature incapable of developing in the dog."

#### V. VICARIOUS EXPERIMENTAL HOSTS OF DIFFERENT STRAINS OF Piroplasma canis.

The first experiments on this subject were those of Lounsbury who, in 1904, was unsuccessful in transmitting the South African piroplasmosis with the tick, Amblyomma hebraeum, and with the Ornithodoros savignyi, and with the lice found on dogs Pulex serraticapsa.

Later Christophers (1907) was unable to transmit Indian piroplasmosis to two dogs with the larvae of female Rhipicephalus bursa, nourished on infected dogs, nor was he successful with two other dogs bitten by adult daughter-ticks, from the same source.

In 1919, in my two articles (a and b) I made various attempts with several strains of canine piroplasmosis. It turned out that 34 Ixodes ricinus nymphs and 387 Haemaphysalis concinna, coming from females gorged with virulent blood were unable to transmit the infection to a dog. This was not surprising since Rh. sanguineus and D. reticulatus nymphs had also produced negative results.

In 1922, I established, with F. Larousse, the possibility of transmitting a Paris strain of canine piroplasmosis from daughter-ticks of D. andersoni (i.e., D. venustus) coming from four females gorged on a dog infected by inoculation of blood from a chronic animal carrier of the germs. Experiments with larvae and nymphs have not been undertaken.

#### VI. DISCUSSION

The experiments covered in this article show how varied the results have been with different authors studying the transmission of canine piroplasmosis by vicarious or natural vectors. This fact should make us wary of generalizing from the small body of experimental results, which we actually possess on this subject.

Do the results obtained by the authors prove that certain strains of canine piroplasmosis are able to present infectious metacyclic forms among nymphs and certain Ixodidae? Contrariwise, do these findings prove an aptitude on the part of nymphs and different breedings of Ixodidae to permit a complete evolution of Piroplasmae of any strain and an orientation of infectious forms toward their salivary glands according to a special attraction? Further experimentation, beyond doubt, will bring elucidation on these problems.

On the other hand, in the case of infections from stage to stage, I think it useful to raise the possibility of unsuspected hereditary infections among the Ixodidae considered as "new", and as a result, the necessity of

establishing the exact genealogy of the ticks used (just as for the control experiments done on new dogs) in order to make sure that the ticks used are without any infection.

In the case of bovine theileriasis, where parasitic relapses are observed spontaneously without any possible artificial or natural reinfection (Brumpt, 1942), a homologous strain may, by an inoculation of blood, give a new infection, generally attenuated, in a large percentage of cases. With heterologous viruses, also transmitted by inoculation of blood, the percentage is much higher and mortalities are far from rare. (Adler and Ellenbogen, 1935). However the results are quite different when the animals infected by a particular virus are exposed to the natural virus transmitted by the ticks. The most important determinations on this subject have been furnished by E. Sargent and his associates (1935), who have established that for 14,000 Algerian cows vaccinated with a very weak virus (Kouba virus), the breeders and the veterinarian only registered 61 deaths (14), which is .43 percent. It can be affirmed that these animals, distributed throughout North Africa, were certainly bitten by ticks carrying germs belonging to perhaps very pathogenic strains.

It would be interesting to do research, on a large number of spontaneously parasited animals in an endemic zone, to find out the percentage of deaths from theileriasis when they are transferred into other zones equally infected with theileriasis. It is true that this percentage could be debated, since it is known that relapses are often observed in this disease during long changes in beasts suffering from an insufficient diet.

On the subject of crossed immunity by the heterologous infections, I have an exact and private experimental document. A Salers heifer was infected June 16, 1937, at Paris, with adult Hyalomma mauritanicum (15) raised from the larvae and nymphal stage on another Salers animal (968/XXII), presenting theileriasis (T. annulata) from Iran, this strain having been sent to me by M. Delpy. This heifer (150/XXIII) presented a fever from the 13th to the 24th day and a sufficiently intense infection of the blood. On July 10, 1937, when the animal was clinically cured, but still presenting parasites, quite rare though, in the peripheric blood, a control experiment (477/XXIII) was done with 42 adults of Hyalomma mauritanicum infected with North African theileriasis (16).

At no time did this heifer have any particular parasitic or thermal reaction and it seemed surely that the strain from Iran had conferred a perfect immunity against the Algerian strain, which entitles us to consider them as identical.

As I mentioned in 1913, concerning Trypanosoma cruzi, the experiments done with metacyclic forms of invertebrate vectors and those done with sanguicolous forms, give quite different results. That is why, in spite of the clinical and experimental observations of M. Carpano (1930) and various authors, with infection of the blood, we do not believe, at least as far as the bovines are concerned, in the possibility of a superinfection under natural conditions. Often the mortal infections observed, can be attributed

to relapses, determined by factors sometimes known and very often unknown.

## VII. CONCLUSIONS

From the proofs of crossed artificial immunity, by inoculation of virulent blood, and natural crossed immunity, by the host vectors themselves, it is found that the strains of canine piroplasmosis seem to possess different antigenic properties, even when the morphology of the parasites which cause them is identical and when their host vectors are the same. This fact has led various authors to admit the existence of different races or even different species.

One certain fact is that the canine Piroplasmae behave differently from the bovine Piroplasmae and Anaplasmae which according to my numerous experiments belong to a group which confers a solid immunity against homologous or heterologous viruses, which are transmitted to them either by inoculation of blood or from the bites of ticks. The bovines hardly ever, after strong doses of a heterologous virus, present even a light febrile or parasitic infection. Furthermore this strong premonition is the basis for the vaccination used by breeders of bovines destined for export into places where the various Piroplasmae cause more or less considerable damage.

## ABSTRACT

1. -- Since the study of canine piroplasmosis has, besides its practical importance, a great scientific interest in that it concerns particularly the problem of the identification of species morphologically identified with the protozoan, acquired active immunity, conditions determining the virulence of infectious germs, and finally the action of host vectors on the virulence of parasites, I have thought it useful to summarize the experiments done on this subject.
2. -- It is possible to transmit from the nymphal to the adult stage, the French piroplasmosis from around Paris, by the South African tick Haemaphysalis leachi.
3. -- This same tick was not able under the same conditions to transmit the P. gibsoni.
4. -- Haemaphysalis leachi adult daughter-ticks, coming from females nourished on a dog having French piroplasmosis from the Paris locality, were not able to infect a new animal.
5. -- Proofs of artificial crossed immunity by inoculations of blood establish the different antigenic properties of several strains of P. canis.
6. -- Proofs of natural crossed immunity by bites of ticks infected with the Piroplasmae which they transmit naturally, show the possibility of new infections, sometimes less severe than among the new controls. These reinfections establish the absence of premonition against heterologous strains, which can lead to death.

7. -- These facts are all the more curious since they differ completely from those which have been established in the case of bovine piroplasmoses and anaplasmoses, where a solid polyvalent immunity by inoculation of virulent blood, or after being bitten by numerous ticks infected with known viruses, is observed among the animals in a state of natural premunition or artificial premunition by vaccination.

8. -- The absence of polyvalent immunity, in the case of canine piroplasmosis, brings it into harmony with that which is observed in the bovine theileriasis caused by Theileria annulata, more or less as concerns the proofs of artificial crossed immunity by inoculation of blood. On the other hand, this theileriasis is distinguished from canine piroplasmosis by the fact that the vaccination of bovines with a weak virus confers a very strong immunity to animals exposed to the bites of ticks, harboring more or less virulent heterologous strains of T. annulata. This observation is confirmed by the success of vaccinations in North Africa and in Palestine.

The polyvalent immunity in the case of Theileria annulata is further established by my own experiment mentioned in this article; it was done with a cow infected by ticks with a strain of T. annulata from Iran and not having reacted to the bites of other ticks which were infected with an Algerian strain of the same species of Theileria.

9. -- The experiments published in this work do not permit us to take a firm position on the existence of several different species, presently grouped under the name Piroplasma canis.

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- (1) For a year I turned over the breeding of the ticks at my laboratory to M. Marchaud, who was studying in the natural sciences.
- (2) We again point to the immunity conferred by the species of Plasmodium from paludism against the heterological strains from different parts of the world where the germs are nonetheless transmitted by about seventy species of anopheles divided into several different genuses and living in the most varied climates.
- (3) Studied by Mathis and Leger, and very probably transmitted by the tick Rh. sanguineus, which is common on the dog throughout the temperate and tropical Far East, this Tonkinan piroplasmosis was sent in May 1909 to the laboratory of F. Mesnil at the Institut Pasteur de Paris, where it has been preserved by passages in dogs by several writers (Leboeuf, Ringenbach, Guica, etc.) In 1911, it had reached the 43rd passage.

(4) To be able to prove the existence of a state of premunition, a new dog should be infected with the blood of this animal, which has not been done. According to my observations, in cases with the French strain of piroplasmosis, the blood sometimes loses its infectious power in three or four months.

(5) Here are the details of the experiment. The dog was 4 weeks old when the first application of ticks was made, and 8 weeks old for the second experiment. The third infection by the rhipicephales was when the animal was 14 weeks old. The resulting infection was progressively weaker and during the third parasitic reaction, the number of Piroplasmae was much less higher than a control dog of the same age.

(6) The unfertile females gorge themselves incompletely and much more slowly than the fertile ones. Dog 66/XXIV, as dog 186/XXIV which served as a control for this experiment, had been infected with some females, for the purpose of observing the partheno-tropical diapause which I have studied for a long time among the ixodines.

(7) This strain came from Paris. It was sent to Professor Mayer by Professor Mesnil, to whom I always furnished the strains of canine piroplasmosis for the courses in bacteriology at the Institut Pasteur. Since, in 1928, I had a strain from a spontaneous case sent from Alfort by Professor Panisset, isolated Nov. 5, 1927 and lost in August 1929, it is probable that it is this virus which has been used by the Holland authors and which is still preserved by Professor Kikuth at Elberfeld.

(8) It is impossible to admit that the rhipicephales of Java were incapable of transmitting the Piroplasmae since Regendanz and Reichenow (1932) using ticks of the same breeding, have succeeded in transmitting a canine piroplasmosis of the Far East, determined probably in nature by the tick Rh. sanguineus

(9) As, according to the work of Swaminath and Shortt (1937), the incubation of the infection of P. gibsoni, transmitted in the Indies from stage to stage and hereditarily, by the tick Haemaphysalis bispinosa, requires 12-18 days, our experiment can be considered negative.

Discovered Sept. 26, 1934, at Hangchow (near Shanghai, China) by Dr. G. Rose on a mongrel (whose number was 3,679), this Chinese virus was inoculated into another dog (4,226) entrusted later to Dr. Vogel, who returned to Hamburg. After two passages made during the trip, the strain was maintained at the Institut des Maladies Tropicales, where, since 1934, it has been transmitted from dog to dog. It is thus possible that it has lost its power to accomplish a cyclic evolution among ticks.

We thank Professors Mühlens and Reichenow, who willingly supplied us with this virus.

(10) This strain isolated in 1934, imparted by four D. reticulatus females, and obtained by Mlle. Martin from a dog having canine piroplasmosis, is always conserved at my laboratory in ticks. The percentage of infected nymphs, male or female, is nearly 100%.

(11) Since Lounsbury, in the fundamental studies that he has made on the South African canine piroplasmosis, notes that neither the larvae nor the nymphs of H. leachi, nourished on the sick animals, have contracted the infection, we have started some experiments (520/XIV) with new nymphs of this species and the South African virus conserved in ticks at my laboratory, which will be published later.

(12) This negative experiment, refixing the ticks, certain ones of which have more or less transmitted French piroplasmosis to dog 288/XIV, did not surprise us. Lounsbury (1904) has published four negative experiments with a South African strain in transplanting respectively 10, 50, 60, and 50 males to four new dogs. The only positive result was obtained in transplanting 20 males bred from infected tick-mothers. Lounsbury notes elsewhere that the smallest number of ticks which gave a dog an infection was 10.

The H. leachi seem in other respects, according to my experiments and histological studies, to be less effective vectors than the Rh. sanguineus and especially the D. reticulatus, which in certain lots are infected 100 out of 100 cases. In the short article which I devoted to the study of the evolution of P. canis (1937), I pointed out that it had been impossible to observe evolving forms of this parasite among the ticks H. leachi and Rh. sanguineus. Very recently, however, I had the good fortune to observe a female of the latter species (which I have inoculated with a tick coelomic trypanosome) strongly parasited by all the evolutionary stages of P. canis (Moroccan strain).

(13) In using another strain of French canine piroplasmosis from the East of France, which I finally isolated in working with D. reticulatus (strain 832/XI of May 7, 1930), Regendanz and Reichenow (1932), to whom I sent this virus, were not successful in infecting a dog by bites of larvae coming from infected female ticks, but they did obtain infections with the nymphs coming from these larvae.

(14) Furthermore certain of these deaths might be attributed to relapses.

(15) Graciously collected at my request by M. Velu from bovines in Morocco.

(16) These ticks, for which I am obliged to Ed. Sergent, were infected in the larvae and nymphal stage in December 1936, on calf L.45 at the Institut Pasteur d'Algérie.

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Item from the News Notes on the Culture of Piroplasma canis.

by H. Ziemann.

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Bull Soc Pathol Exot 6:140-141, 1913.

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Our colleague, Professor H. Ziemann, sends to the society some galley proofs of two works on the culture of malarial parasites, and he announces that he has been successful in cultivating this parasite in a case of malignant tertian fever in Cameron.

M. Ziemann also announces that he has obtained cultures of second generation Piroplasma canis. The technique used is similar to the one extolled by Bass. Ziemann offers the following considerations: 1st, that the optimum temperature for this culture is 37°; 2nd, that, for the subcultures, it is good to warm the dog serum for one hour at 45°, to inactivate it; that one should add to the inactivated and dextrosed serum (0 cc, 1 of a 50 % dextrose solution, for 8 cc. of blood with Piroplasmae) some physiological water, citrated to 2 %, in the proportion of 0 cc., 3 to 0 cc., 4, for 10 cc. of inactivated dog serum. The elimination of the leucocytes by centrifugation is done according to Bass's method. One obtains in this manner, in the culture liquid, some fine forms of multiplication, with 16 malarial parasites or better, as is never or almost never seen in the peripheral blood. The cultures kept at 40°, and those kept at laboratory temperature give slightly less, but equally fine results. The growth of the cultures stops after about 48 hours; if the cultures are kept at 40°, the growth is very rapid, but the degeneration is equally rapid.